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(54) ESCHERICHIA COLI BETA GENE, PLANT OF FAMILY GRAMINEAE TRANSFORMED WITH THE GENE AND PRODUCTION OF THE PLANT

(57)Abstract:

PROBLEM TO BE SOLVED: To obtain the subject new gene producing Oryza sativa having resistance to higher salts or drying by removing a poly(A) addition signal-like sequence and a palindrome sequence from a gene coding a natural-type Escherichia coli betA protein.

SOLUTION: This new gene has a base sequence obtained by removing a poly(A) addition signal-like sequence and a palindrome sequence from a base sequence of a gene coding a natural-type Escherichia coli betA protein and codes the Escherichia coli betA protein. The gene highly manifests in a plant of family gramineae and the plant of family gramineae transformed by introducing the gene produces and accumulates glycine betaine, then the utility as a crop having resistance to higher salts stress or drying stress can be expected. The gene is obtained by collecting a gene coding a natural-type Escherichia coli betA protein and modifying its base sequence by removing a poly(A) addition signal-like sequence and a palindrome sequence by using a site-specific variation method.

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* NOTICES *

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DETAILED DESCRIPTION

[Detailed Description of the Invention]
[0001]
[Field of the Invention] This invention relates to the manufacture approach of of the salt atmosphere and the drought-resistant grass by introducing the synthetic gene which carries out the code of the biosynthesis enzyme protein of the glycine betaine (it abbreviates to a "betaine" hereafter) known as an adaptation solute to a detail, and the gene concerned about the grass which introduced the adaptation solute synthetic gene of the Escherichia coli origin, and this gene, and was obtained, and its manufacture approach.
[0002]
[Description of the Prior Art] In recent years, research on an adaptation solute (a low-molecular organic compound, osmotic-pressure pacemaker) is actively advanced as a device in which a living thing is adapted for a high salts environment or desiccation. For example, as an adaptation solute, by carrying out high concentration are recording of the betaine of the 4th class ammonium compound, or the proline of amino acid intracellular, Escherichia coli raises intracellular osmotic pressure and is adapted for an environment under a high salts environment. Moreover, the salt atmosphere and drought-resistant vegetation also accumulated the 3rd class sulfonium compound like a betaine, a proline, or dimethyl SURUFONIO propionate, sugar-alcohol like a mannitol, etc. in cytoplasm, and became clear [that it is adapted for salts stress or desiccation stress]. On the other hand, it is thought that the corn of the important crops of Poaceae, for example, a rice, and a spread kind etc. is weak to a high salts environment or desiccation since the capacity which accumulates these adaptation solutes is lacked.
[0003] Also in the adaptation solute, the betaine is used by many vegetable specieses, and the elucidation of functions other than vegetable intracellular osmoregulation, i.e., the protective action of the protein of a chloroplast, (Nomura et al., the collection of the Japanese Society of Plant Physiologists annual convention lecture summaries in the 1996 fiscal year, p146) is progressing. Then, the research on the breeding of the salt atmosphere and the drought-resistant crops by introducing the gene of the enzyme protein in connection with a betaine biosynthesis into vegetation without betaine productivity is becoming prosperous. For example, woods carried out cloning of the choline oxidase gene which an Actinomyces has, and were made to discover it by Arabidopsis, and it has succeeded in obtaining transformant vegetation with betaine productivity (the collection of the Japanese Society of Plant Physiologists annual convention lecture summaries in the 1996 fiscal year, p146). Although choline oxidase is an enzyme converted into a betaine by the single step from the choline which is a precursor, there is a problem of carrying out equivalent production of the toxic high hydrogen peroxide with a betaine extremely for a plant cell, in the case of the reaction.
[0004] In Escherichia coli, a betaine is produced with two enzymes, the choline dehydrogenase converted into the betaine aldehyde from a choline, and the betaine-aldehyde dehydrogenase converted into a betaine from the betaine aldehyde. Although the code of the choline dehydrogenase is carried out to the betA gene, a certain thing is reported also for betaine-aldehyde dehydrogenase activity to the protein by which a code is carried out to this gene. Nomura and others introduced the betA gene of

Escherichia coli into freshwater blue-green algae, and succeeded in salt atmosphere strengthening (Plant Physiol.107,703-708 (1995)). Moreover, it is reported that Lilius and others got the transformant vegetation of stress resistance by introducing a betA gene into tobacco (Bio/Technology, 165 (3), and 849 (1996)). However, there is no proof that the betA gene was discovered in the plant cell, and it cannot be proving that the betaine was accumulated, either. Furthermore, on agriculture, introduce a betA gene, it is made discovered in an important gramineous crop, and the report of having obtained the transformant with betaine production ability is not yet made.
[0005]
[Means for Solving the Problem] this invention persons found out that the array which is not desirable to the gene expression in vegetation, especially a grass existed in a gene sequence, as a result of analyzing a betA gene in a detail. That is, they are six poly(A) addition signal Mr. arrays which bar a manifestation on ** imprint level, and the palindromic sequence which bars a manifestation on ** translation level. Furthermore, in the betA gene, it found out that the codon hardly used existed with the grass gene. Then, as a result of considering the manifestation by the grass of the gene concerned, a specific alteration gene came to complete a header and this invention for it being high-discovered by the grass with transgenics, carrying out the code of the enzyme protein of a betA gene product, storing up a betaine into a plant body, and getting for the first time.
[0006] That is, in case gene expression of the summary of this invention is carried out in vegetation, it consists in the grass by which the transformation was carried out by the vector into which the betA gene (henceforth an "alteration mold betA gene") changed so that the poly(A) addition signal Mr. array in the gene concerned used as hindrance and a palindromic sequence might be lost, and the gene concerned were introduced, and the vector concerned, and its manufacturing method.
[0007] In this invention, the target vegetation is a grass, and especially a limit will not be carried out if it belongs to Poaceae. A rice, wheat (wheat, a barley, rye wheat, etc.), Japanese millet, a foxtail millet, lawn grass, corn, etc. are specifically mentioned, and a rice is desirable especially in this invention.
[0008]
[Embodiment of the Invention] Hereafter, it explains to a detail per this invention.
[0009] The alteration mold betA gene of alteration mold betA gene this invention of <1> this invention is a gene which has the base sequence from which the poly(A) addition signal Mr. array and the palindromic sequence were removed from the base sequence of the gene which carries out the code of the natural mold Escherichia coli betA protein, and carries out the code of the Escherichia coli betA protein. As a poly(A) addition signal Mr. array, ATTATT, AATAAC, TTTATT, AATATT, ATTAAC, and TATAAC are mentioned, and TGCCGGCTCAGCCGGCA is mentioned as a palindromic sequence.

[0010] The gene which carries out the code of the natural mold Escherichia coli betA protein is indicated by Molecular Microbiology, 5 (5), and 1049 (1991). The base sequence is an array as shown in the array number 2 of an array table, and is carrying out the code of the protein which has the amino acid sequence shown in the array number 3. In addition, in the base sequence shown in the array number 2, the 6 base GGATCC like the BamHI cutting section is added before a translation initiation codon, the 6 base GAGCTC like the SacI cutting section is respectively added after 27 bases of a codon from beginning to end, and the translation initiation codon is permuted by ATG from TTG.
[0011] The gene of this invention is changed chemically and in enzymology so that the code of betA protein and the protein equivalent on a functional target may be carried out and it may be discovered on level higher than a betA gene natural within a grass. Here, betA protein and protein equivalent on a functional target are protein which has the activity which 1 or some amino acid consist of deletion and an amino acid sequence permuted or added in the protein in which a betA gene carries out a code, i.e., the protein which has the amino acid sequence shown at the array number 3 of an array table, and this amino acid sequence, and generates a betaine from a choline. DNA which carries out the code of such betA protein and the protein equivalent on a functional target is acquirable by the site-specific mutation method based on for example, a wild type betA gene. Moreover, the gene which has deletion and the base sequence permuted or added can be obtained also by choosing the protein which has the activity

which is DNA which has the base sequence of a publication for the array number 2, and DNA hybridized under stringent conditions, and generates a betaine from a choline from DNA obtained by performing variation processing into the cell which has a wild type betA gene or this, or a cell microorganism. "Stringent conditions" here means the conditions in which the so-called specific hybrid is formed in and a nonspecific hybrid is not formed. Although it is difficult to evaluate this condition clearly, if an example is shown, nucleic acids with high homology, for example, DNA which have 80% or more of homology preferably 60% or more, will hybridize, and the conditions which nucleic acids with homology lower than it do not hybridize will be mentioned.

[0012] Without changing these amino acid sequences to the amino acid sequence or betA protein in which a natural betA gene carries out a code, and a functional target based on the amino acid sequence of equivalent protein, the alteration mold betA gene of this invention is changed so that only the class of codon of each amino acid may be changed. Specifically, it is changed so that the following two points may be fulfilled.

[0013] ** It is other arrays which do not serve as hindrance of an imprint in the poly(A) addition signal Mr. array in the natural betA gene used as the hindrance of a gene imprint, and permute by array which does not cause amino acid substitution. As an array after a permutation, the array specifically shown in Table 1 is mentioned. These poly(A) addition signal Mr. arrays strike, and at least one all [two or more] are permuted especially preferably.

[0014]

[Table 1]

置換前の配列	配列番号２における位置	置換後の配列	配列番号１における位置
ATTATT	25 ～ 30	ATCATC	19 ～ 24
AATAAC	223 ～ 228	AACAAC	217 ～ 222
TTTATT	1048 ～ 1053	TTCATC	1042 ～ 1047
AATATT	1081 ～ 1086	AACATC	1075 ～ 1080
ATTAAC	1111 ～ 1116	ATCAAC	1105 ～ 1110
TATAAC	1117 ～ 1122	TACAAC	1111 ～ 1116

[0015] ** Permute palindromic sequence TGCCGGCTCAGCCGGCA in the natural betA gene used as the hindrance of a translation of a gene (it sets for the array table array number 2, and they are the base numbers 33-49) by an array from which the permutation of the amino acid which forms and carries out the code of the palindrome does not arise, for example, TGCCGGGTCAGCGGGCA, (it sets for the array table array number 1, and they are the base numbers 27-43).

[0016] The above permutation mold betA genes are acquirable by performing the magnification reaction by the polymerase chain reaction method (it abbreviating to "PCR" hereafter (Am.J.Hum.Genet., 37, 172, 1985)) using the synthetic single-strand oligonucleotide (it abbreviating to a "primer" hereafter) of 63 including the field which is going to use for example, a natural mold betA gene as mold, and is going to permute it - 85 base extent. composition of an oligonucleotide -- a law -- when the codon activity ratio in a grass of the codon of the amino acid in a primer is 1% or less in that case, it is [that what is necessary is just to carry out by the method] desirable to change into codons with a high activity ratio, such as a codon of the maximum activity ratio. What was designed so that the above-mentioned permutation might be included should just be used for the primer used for PCR based on the base sequence of a betA gene. The array of a primer and the method of acquiring the alteration mold betA gene by the PCR method are illustrated below.

[0017] First, on both sides of the array of about 300 bp including the base sequence which wants to

change a five prime end part among the arrays of a natural mold betA gene, 5'3 of side primer ** (array number 6) and 63 bp' side primer ** (array number 7) of 63 bp is compounded. The double stranded DNA (in the array number 4, it is equivalent to the base numbers 1-275 in 7-290, and the array number 1) is amplified by PCR by using a natural betA gene as mold. Hereafter, this DNA fragment is called "DNA fragmentA." In addition, the base sequence which carries out the code of a part of chloroplast shift array of a glutamine synthetase, and the recognition sequence (GGATCC) of a restriction enzyme are added to the upstream of an initiation codon at the five prime end side of primer **.

[0018] Next, two primers ** (array number 9) and primer ** (array number 10) of 85 bp are compounded so that about 15 bp complementary strand may be formed by each three-dash terminal, and the double stranded DNA (in the array number 5, it is equivalent to the base numbers 1010-1165 in 78-233, and the array number 1) of 156 bp is amplified by PCR using this primer. Next, an PCR reaction is performed using obtained DNA and primer ** (array number 8), and the double stranded DNA (in the array number 5, it is equivalent to the base numbers 940-1165 in 8-233, and the array number 1) of 226bp is obtained. Hereafter, this DNA fragment is called "DNA fragmentB."

[0019] Cloning of DNA fragmentA and DNA fragment B which are obtained as mentioned above is carried out to the Escherichia coli vector which has a multiple cloning site, for example, pCRTMII, (the product made from Invitrogen, TA Cloning Kit). Then, each DNA fragment by which cloning was carried out is determined and checked in an array by the dideoxy chain termination method of Sanger et al., (Proc.Natl.Acad.Sci., 74, 5463-5467, and 1977) etc. each DNA fragment -- a law -- it becomes the structural gene of the overall length which finally carries out the code of the betA protein by replacing with the part to which each DNA fragment after cutting and in a natural betA gene corresponds with a restriction enzyme using the restriction enzyme part which it has to both ends by the method. The example is shown in the array number 1 of an array table. In addition, in this base sequence, as shown in Table 2, the codon with low operating frequency is permuted by the codon of other equivalence with the grass gene, so that it may not be accompanied by the permutation of amino acid. In this invention, 18 or more all [20 or more] are permuted especially preferably preferably among these codons.

[0020]

[Table 2]

表2

コドンの位置	置換前のコドン	置換後のコドン
3	TTT	TTC
80	GGA	GGC
82	GGT	GGC
83	AAA	AAG
86	GGT	GGC
87	GGA	GGC
325	AAA	AAG
327	GGT	GGC
332	TTT	TTC
335	ACT	ACC
337	GTT	GTC
338	GGT	GGC
343	TTT	TTC
344	GAA	GAG
345	GCA	GCG
346	GGT	GGC
347	GGA	GGC
352	CGT	CGC
354	GAA	GAG
363	CAT	CAC
366	CCA	CCG
367	GTA	GTC
375	AAT	AAC
376	GCA	GCC
378	AAA	AAG
381	GGT	GGC

[0021] The vector of vector this invention of <2> this inventions is a vector into which the alteration mold betA gene obtained as mentioned above was introduced. An alteration mold betA gene is included in the plasmid vector which has the intron the promotor and terminator which are discovered in a grass, or if needed.

[0022] As a promotor who uses, the promotor by whom it was checked that it is discovered in vegetation (Science, 244, and 174 (1989)), such as the promotor of the cauliflower mosaic virus origin and rbcS(s) (ribulose1.5-bisphosphate carboxylase), such as CaMV35S (pBI221: EMBO.J., 6, 3901-3907, 1987), and Cab (chlorophyll a/b binding protein), is mentioned. Moreover, it adds to the upstream of a structural gene so that fusion protein with betA protein may be formed for the amino acid sequence (Plant Cell Physiol.34(2):345 (1993)) which makes protein shift to the amino acid sequence (Plant Molecular Biology and 13,611) made to shift to a chloroplast, or (1989) a mitochondrion, and localization of the betA protein is carried out to a chloroplast or a mitochondrion, or it is made for the betA protein discovered without attaching a shift array to exist in cytoplasm.

[0023] As a terminator, the terminator of the cauliflower mosaic virus origin, the terminator of the NOS (nopaline synthesis enzyme) gene origin, etc. are raised, for example. Moreover, between a promotor and a structural gene, the vector which allots the intron can also be used as a high expression vector, and the first intron (Genes&Development, 1, 1183-1200, 1987) of corn Adh1 (alcohol dehydrogenase gene), the first intron (Tanaka et al., Nucleic Acids Research, 18, 6767-6770, 1990) of a castor seed Cat (catalase gene), etc. are raised as the intron, for example.

[0024] It is desirable to use the so-called selective marker gene effective in case two or more foreign

genes further chosen from a hygromycin phosphotransferase gene, a neomycin phosphotransferase gene, a chloramphenicol acetyltransferase gene, a beta-glucuronidase gene, etc. are used and the one target colony is chosen in this invention. As this selective marker gene, a hygromycin phosphotransferase gene is desirable.

[0025] After the grass of <3> this inventions and the grass of that manufacturing method this invention introduce the above-mentioned vector into the protoplast of the grass origin and make a colony form from this protoplast, they reproduce a plant body and are obtained from this colony. Moreover, after the manufacture approach of the grass of this invention suspends the protoplast of the above-mentioned vector and the grass origin in a liquid medium, impresses an electric pulse and introduces this vector, cultivate it by the culture medium containing a rice cultured cell, it makes a colony form, and is characterized by reproducing a plant body from this colony.

[0026] In this invention, what has a selective marker gene and an alteration mold betA gene in the same plasmid may be used, and the plasmid which has a selective marker gene, and the plasmid which has an alteration mold betA gene may be used together. It is used for a vector carrying out the transformation of the grass. That is, after suspending the protoplast of the grass origin in a liquid medium, impressing an electric pulse and introducing the vector concerned, it is the approach of cultivating by the culture medium containing a rice cultured cell, making a colony forming, and reproducing a plant body from this colony (Shimamoto et al., Nature, 337, 274-276, 1989).

[0027] A protoplast can be prepared as follows. For example, after cultivating the suspension cell or callus originating in the organization of the full ripeness of cultivation rices, such as a Japanese fine one, Koshihikari, and sasanisiki, and an unripe seed, a sheath, and a root by the liquid medium, according to a conventional method, enzyme processing is carried out among the enzyme liquid containing cell wall dialytic ferments, such as a cellulase and MASEROZAIMU, for about 3 to 16 hours on condition that 25-30 degrees C and 0 - 50 r.p.m. After enzyme processing termination, it filters, and except for an undigested object, the KMC liquid (0.118M potassium chloride, 0.0817M magnesium chloride, 0.085M calcium chloride, and pH6.0) (63 Theor. Appl. Genet., 53, 57- 1978) of an amount etc. can be added to a filtrate two to 5 times, centrifugal separation can be carried out, and the refined protoplast can be obtained.

[0028] The expression vector containing the alteration mold betA gene prepared as mentioned above, for example, the protoplast of ml, one to 100 microg /, and the above-mentioned vegetable origin, for example, (2-10), x106 piece/ml, is suspended in 30 - 200mM potassium chloride, 0 - 50mM magnesium chloride, 0.2 - 0.6M mannitol, and the buffer solution of pH5.8 containing 0.1%MES, an electric pulse is impressed to this, and a plasmid is introduced into a protoplast. Electric pulse processing is the direct-current pulse of the initial voltage of 200 obtained using a 100-1000-micro F capacitor - 1000 V/cm, and it is suitable for it to impress on condition that pulse width 1 - 50msec extent (refer to JP,1-181791,A).

[0029] The protoplast which carried out electric pulse processing as mentioned above For example, the vitamin mixed liquor of the mineral constituent of R2 culture medium (Plant.Cell.Physiol., 14, 1113, 1973), and MS culture medium (Murashige and Skoog, 15, 473-497, 1962) In the included liquid medium (R2/MS) or MS culture medium, a potassium nitrate is preferably suspended in the culture medium contained 0.2 to 0.5% as a nitrogen source. This is mixed with R2/MS or MS culture medium containing about 1.0 - 3.0% of agarose etc. equivalent [every], and it extends in a petri dish promptly, and hardens thinly. As for the concentration of the protoplast at this time, it is desirable to make it set to abbreviation (5-50) x105 piece/ml.

[0030] Then, the solidified agarose is cut in adult magnitude 5-20mm, and it cultivates on the above-mentioned liquid medium. When the protoplast of the grass origin is used at that time, it cultivates at 23-27 degrees C under a dark condition, carrying out 100-300mgFW / petri dish extent coexistence of the rice cultured cell into a culture medium preferably, and shaking slowly by rotation of 20 - 50r.p.m. The approach of making it coexist with a rice cultured cell puts in the liquid medium containing the protoplast other than the above-mentioned approach in the container which prepared the membrane filter in the bottom, and has a method of dipping the container in the petri dish into which the liquid medium was put with the rice cultured cell, and making it live together. The rice cultured cell shown here has the

desirable object which consists of the fine cell lump divided flourishing. Such a cultured cell carries out the passage of the callus obtained from the seed, the stem, root, or anther of for example, rice vegetation into a liquid medium, and is easily obtained according to the well-known approach of selecting a cell with an early fission rate.
[0031] In three - four weeks, an about 0.5-1mm colony is formed after culture. When a hygromycin phosphotransferase gene (hph) is introduced into a vector as a selective marker gene at that time, if hygromycin will be added in culture medium ml 10-100micro aboutg /on seven - the 20th after culture initiation and culture is continued further, the target transformed cell can be chosen efficiently. Subsequently, it cultivates at 23-27 degrees C under lighting (1,000-4,000lux) for two to four weeks on the agar medium which added plant hormone for this colony and added 2 mg/l extent and agarose for acetic acid (2, 4-D) 0.1 to 1.0% to the growth medium, for example, R2 culture medium, and a callus with a diameter of 3-6mm is obtained. Each callus is separated independently, further, when a hph gene is introduced as for example, a selective marker gene, it ****s to this growth medium containing 20 to 50 microg [/ml] hygromycin, and cultivates, and hygromycin tolerance is checked.
[0032] If this callus is cultivated under 23-27 degrees C and the conditions of 2,000-4,000lux by the R2-/MS culture medium (however, cytokinin hormone additive-free or 1 - 10 mg/l addition) which contains for example, agarose 0.5 to 1.5%, formation of an adventious embryo or an indefinite bud will be accepted in two - ten weeks. An implantable seedling object is acquired by cultivating by the R2-/MS culture medium which does not contain hormone for further two to three weeks. In this way, if the acquired seedling object is transplanted and grown up into a bar MYUKYU light etc., it can obtain the plant body of the rice which is made into the purpose and by which the transformation was carried out.
[0033] That the gene is included in a transformed cell or transformation vegetation isolates these to DNA by for example, Mol.Gen.Genet., 211, 27, and the approach according to 1988, and it can check it with the PCR method (Am.J.Hum.Genet., 37, 172, 1985) or a Southern method (J.Mol.Biol., 98, 505, 1980). Moreover, the thing for which the alteration mold betA gene by which the transformed cell was included in the vegetable genome is discovered For example, the northern method which used the array of an alteration mold betA gene, or its part as the probe (Thomas, P.et al., Proc.Natl.Acad.Sci., 77, 5201, 1980), Western blotting method using the antiserum to the introduced gene product (betA protein) (Towbin et al., Proc.Natl.Acad.Sci., 76, 4350, 1979) It can do clearly. The enzyme activity of the betA protein which is the translation product of the introduced gene can be measured by Nagasawa's and others approach (Agr.Biol.Chem., 40 (10), and 2077 (1976)). The betaine content in a transformation rice plant body is made detection and a quantum by 1 H-NMR according to the approach (Plant Physiol.29 and 1315-1321 (1988)) of Arakawa and others.
[0034]
[Example] Although an example is given per this invention and explained concretely hereafter, unless the summary is exceeded, it is not limited to the following examples.
[0035]
[Example 1] Construction of the expression vector for vegetation containing a non-changed betA gene and a betA structural gene are chemical. With construction cauliflower mosaic virus 35S promotor of the expression vector for vegetation containing the enzymatic synthesis and the joint (1) non-changed betA gene (array table array number 2) to a vector Castor seed The intron of a catalase gene, and DNA which carries out the code of the chloroplast shift array of the glutamine synthetase of a rice, plasmid GSC-GUS (Japan Society for Bioscience, Biotechnology and Agrochemistry and 69 (5) --) which contains a beta-glucuronidase (GUS) structural gene in this sequence, and a chloroplast shift array and the beta-glucuronidase discover in the form of fusion protein 11-13 and (1995) 10 mM Tris-HCl (pH7.5), 7mM MgCl2 and 7mM 2-mercaptoethanol and 20mM KCl (henceforth) After cutting the reaction mixture of this presentation by restriction enzyme SacI 2units in 100microl called Low buffer, 10 mM Tris-HCl (pH7.5), 7mMMgCl2, and 7mM 2-mercaptoethanol and 150mM KCl (henceforth) It cut by restriction enzyme BamHI 2units in 100microl which calls the reaction mixture of this presentation High buffer, and the GUS gene was removed.
[0036] subsequently, a betA gene (Molecular Microbiology and 5 (5) --) The 6 base GGATCC like the

BamHI cutting section is added before the translation initiation codon of 1049 (1991). The betA gene (the translation initiation codon is changed into ATG from TTG) (array number 2 of an array table) which added the 6 base GAGCTC like the SacI cutting section after 27 bases of a codon from beginning to end After cutting by restriction enzyme SacI 2units in Low buffer 100microl, The DNA fragment cut and obtained by restriction enzyme BamHI 2units in High buffer 100microl The ligation kit (Ligation kit and TAKARA SHUZO CO., LTD.) was used for the part which removed the previous GUS gene of plasmid GSC-GUS, it tied to it, and plasmid pbet/chl (drawing 1) was obtained.

[0037] (2) preparation of the primer for PCR -- according to the base sequence (array number 1 of an array table) of the betA gene designed as mentioned above, the oligonucleotide (array numbers 6-10 of an array table) used for the primer for amplifying DNA fragmentA (array number 4 of an array table) and DNA fragment B (array number 5 of an array table) by PCR was compounded. Preparation of an oligonucleotide is Matteucci et al. (1981) It carried out according to J.Am.Chem.Soc.103, 3185-3192 and Beaucage and others (1981), Tetrahedron Lett.22, and the general technique indicated by 1859-1862. All oligonucleotides are Applied Biosystems. It prepared with solid phase phospho friend DAITO triester coupling process using the 391 form DNA synthesizer. Deprotection from the individual support of oligomer and separation were performed using aqueous ammonia according to the standard method 28%. An oligonucleotide purification cartridge (an OPC column, Applied Biosystems) is used for crude oligonucleotide mixture, and it is Mcbridg et al. (1988) Biotechniques, 6; It refined, as indicated by 362-367.

[0038] (3) The location of a codon with low operating frequency is as having been shown in said tables 1 and 2 with the poly(A) addition signal Mr. array by the PCR method which it is going to double-stranded-DNA--ization-permute, and a grass gene. Moreover, a palindromic sequence exists in the base numbers 33-49 (TGCCGGCTCAGCCGGCA) in the array table array number 2.

[0039] DNA fragment A is the fragment of 284bp(s) which has BamHI and a BclI part in both ends, and this was amplified by performing an PCR reaction by using a natural betA gene as mold as mentioned above, using two oligonucleotide **** (array numbers 6 and 7 of an array table) as a primer (array number 4 of an array table). PCR Primer concentration 10microM, 10 mM Tris-HCl (pH 8.3), 1.5mM MgCl2, 50mM KCl, and 0.005% NP-40 and 0.001% Gelatin, dATP, dGTP, dCTP, dTTP, Reaction mixture of every 200microM, AmpliTaqR DNA polymerase (PERKIN-ELMER) 5 units, and all 100microl (in double-stranded-DNA-ization by the PCR method in the term of (3), henceforth) except that the class and template DNA of a primer differed from each other, it carried out using the reaction mixture of the same presentation. Using DNA thermal SAIKURA (MJ RESEARCH), the repeat reaction was carried out 30 times and 94 degrees C, 1 min., 54 degree C, 2 min., 72 degree C, and the cycle that consists of 3 min. were performed.

[0040] DNA fragment B is the fragment of 226bp(s) which has PstI and a BglI part in both ends, and this was amplified by PCR which used three oligonucleotide primer ****** (array numbers 8, 9, and 10 of an array table). DNA fragment B performed PCR using primer ** and ** first, and compounded DNA of 156bp. On condition that 94 degrees C, 1 min., 45 degree C, 1 min., 72 degrees C, and 2 min., repeatedly, PCR carried out the repeat reaction 20 times next on condition that 94 degrees C, 1 min., 60 degree C, 1 min., 72 degree C, and 2 min., and was performed 5 times. Then, PCR was performed by the same reaction condition as the 1st time using DNA5microl and primer ** of 156bp(s) which are the above, and were made and amplified, and DNA of 226bp(s) was compounded (array number 5 of an array table).

[0041] Construction of a plasmid was performed according to drawing 1 -4. Plasmid pbet/chl 10 mM Tris-HCl (pH7.5), 7mM MgCl2, 7mM 2-mercaptoethanol, and 60mM NaCl (the reaction mixture of this presentation is henceforth called Medium buffer) After [High] cutting by restriction enzyme BclI 2units in 100microl It cuts by restriction enzyme BamHI 2 units in buffer 100microl, and they are Seakem GTGAgarose (FMC company) and 1xTBE 0.7%. Electrophoresis is carried out with the buffer solution and it is 4.9 kbp. The DNA band was cut down. This band is put into a dialysis tube and it is 0.5xTBE. In the buffer solution, DNA was migrated and 135mA constant current was collected from a sink and gel. They are 40microl3M NaOAc and 1000microl 100% to the DNA solution of obtained

400microl. Ethanol is added, centrifugal is carried out by 15000 rpm for 15 minutes, and it is the target 4.9 kbp. DNA fragment C (drawing 1) was refined.
[0042] Subsequently, after cutting Escherichia coli vector pCRTMII/A in which DNA fragment A was inserted by restriction enzyme BclI 2 units in Medium buffer100microl, it cuts by restriction enzyme BamHI 2 units in High buffer 100microl, and they are NuSieve GTG Agarose (FMC company) and 1xTBE 4%. Electrophoresis was carried out with the buffer solution and the DNA band of 278 bp was cut down. This band is put into a dialysis tube and it is 0.5xTBE. In the buffer solution, DNA was migrated and 135mA constant current was collected from a sink and gel. They are 40microl 3M NaOAc and 1000microl 100% to the DNA solution of obtained 400microl. Ethanol is added, centrifugal is carried out by 15,000 rpm for 15 minutes, and it is the target 278 bp. DNA fragment A' (drawing 1) was refined. each refined DNA -- about -- 1/10 amount -- T-four DNA DNALigation Kit (Takara) using a ligase -- using -- the manual of a kit -- following -- all -- 50 It joined together by the system of reaction of mul, and the strain into which plasmid pbet/chl/M1 (drawing 1) which the DNA fragment A phase this part replaced by carrying out a transformation to Escherichia coli stock DH5alpha, and selecting by the chloramphenicol was introduced was obtained. This bacillus was cultivated and plasmid pbet/chl/M1 was refined.
[0043] After cutting plasmid pbet/chl/M1 by restriction enzyme SacI 2 units in Low buffer 100microl, it cuts by restriction enzyme SalI 2 units in High buffer 100microl, and they are Sea kem TG Agarose (FMC company) and 1xTBE 1%. Electrophoresis is carried out with the buffer solution and it is 1.9 kbp. A DNA band is cut down, and it puts into a dialysis tube, and is 0.5xTBE. In the buffer solution, DNA was migrated and 135mA constant current was collected from a sink and gel. They are 40microl 3M NaOAc and 1000microl 100% to the DNA solution of obtained 400microl. Ethanol is added, centrifugal is carried out by 15,000 rpm for 15 minutes, and it is the target 1.9 kbp. DNA fragment D (drawing 2) was refined. 2.9 kbp similarly cut and refined with restriction enzymes SalI and SacI about the Escherichia coli vector pBluescriptRII KS (STRATAGENE) DNA fragment E (drawing 2) was obtained. It is T-four DNA about the ten refined amounts of about 1/of each of DNA. According to the manual of a kit, it joined together by the system of reaction of all 50microl using DNA Ligation Kit (Takara) using a ligase, and the strain into which plasmid pBS/bet/M1 (drawing 2) in which the betA structural gene part mutated by carrying out a transformation to Escherichia coli stock DH5alpha, and selecting by ampicillin was inserted was introduced was obtained. This bacillus was cultivated and plasmid pBS/bet/M1 was refined.
[0044] It is Low buffer 100 about plasmid pBS/bet/M1. After [High buffer / 100] cutting by restriction enzyme SacI 2 units in mul It cuts by restriction enzyme PstI 2 units in mul. 1% Sea kemGTG Agarose (FMC company) and 1xTBE Electrophoresis is carried out with the buffer solution and it is 4 kbp. A DNA band and the DNA band of 742 bp are cut down. It puts into a dialysis tube, respectively and is 0.5xTBE. In the buffer solution, DNA was migrated and 135mA constant current was collected from a sink and gel. They are 40microl 3M NaOAc and 1000microl 100% to the DNA solution of obtained 400microl. Ethanol is added, centrifugal is carried out by 15,000rpm for 15 minutes, and it is the target 4 kbp. DNA fragment F (drawing 3) and 742 bp DNA fragment G (drawing 3) was refined. 742 The PstI-SacI fragment G of bp is High buffer 100 further. It cuts by restriction enzyme BglI 2 units in mul, and they are NuSieve GTG Agarose (FMC company) and 1xTBE 4%. Electrophoresis was carried out with the buffer solution, the band of 542 bp was cut down, and it put into the dialysis tube, and in the 0.5xTBE buffer solution, DNA was migrated and 135mA constant current was collected from a sink and gel. They are 40microl 3M NaOAc and 1ml 100% to the DNA solution of obtained 400microl. Ethanol is added, centrifugal is carried out by 15,000rpm for 15 minutes, and it is the target 542 bp. DNA fragment H (drawing 3) was refined.
[0045] Subsequently, it is Highbuffer 100 about Escherichia coli vector pCRTMII/B (drawing 3) in which DNA fragment B was inserted. After [High buffer / 100] cutting by restriction enzyme PstI 2 units in mul It cuts by restriction enzyme BglI 2 units in mul. Electrophoresis is carried out with NuSieve GTG Agarose (FMC company) and the 1xTBE buffer solution 4%, and it is 205 bp. A DNA band is cut down, and it puts into a dialysis tube, and is 0.5xTBE. In the buffer solution, DNA was

migrated and 135mA constant current was collected from a sink and gel. They are 40microl 3M NaOAc and 1ml 100% to the DNA solution of obtained 400microl. Ethanol is added, centrifugal is carried out by 15,000 rpm for 15 minutes, and it is the target 205 bp. DNA fragment B' (drawing 3) was refined.
[0046] 205 BglI-SacI Fragment H of PstI-BglI Fragment B' of Bp, and 542 Bp T-four DNA DNA Ligation Kit (Takara) using a ligase -- using -- the manual of a kit -- following -- all -- 50 Ethanol precipitate refines, after joining together by the system of reaction of mul. subsequently, DNA fragment F of it and 4 kbp -- T-four DNA DNA Ligation Kit (Takara) using a ligase -- using -- the manual of a kit -- following -- all -- 50 It joined together by the system of reaction of mul. The strain into which plasmid pBS/bet/M2 (drawing 3) in which the betA structural gene part into which variation went by carrying out a transformation to Escherichia coli stock DH5alpha using this reaction mixture, and selecting by ampicillin was inserted was introduced was obtained. This bacillus was cultivated and plasmid pBS/bet/M2 was refined.
[0047] After cutting plasmid pBS/bet/M2 by restriction enzyme SacI 2 units in Low buffer 100microl, it cuts by restriction enzyme SalI 2 units in High buffer 100microl, and they are Sea kem GTG Agarose (FMC company) and 1xTBE 1%. Electrophoresis is carried out with the buffer solution and it is 1.9 kbp. A DNA band is cut down, and it puts into a dialysis tube, and is 0.5xTBE. In the buffer solution, DNA was migrated and 135mA constant current was collected from a sink and gel. They are 40microl 3M NaOAc and 1ml 100% to the DNA solution of obtained 400microl. Ethanol was added, centrifugal was carried out by 15,000 rpm for 15 minutes, and DNA fragment I (drawing 4) of the purpose was refined.

[0048] After cutting plasmid pbet/chl by restriction enzyme SacI 2 units in Low buffer 100microl, it cuts by restriction enzyme SalI 2 units in High buffer 100microl, and they are Sea kem GTGAgarose (FMC company) and 1xTBE 1%. Electrophoresis is carried out with the buffer solution and it is 3.5 kbp. A DNA band is cut down, and it puts into a dialysis tube, and is 0.5xTBE. In the buffer solution, DNA was migrated and 135mA constant current was collected from a sink and gel. They are 40microl 3M NaOAc and 1ml 100% to the DNA solution of obtained 400microl. Ethanol was added, centrifugal was carried out by 15,000 rpm for 15 minutes, and DNA fragment J (drawing 4) of the purpose was refined.
[0049] 1.9 of the betA structural gene containing variation It is T-four DNA about the kbp SalI-SacI fragment I and the 3.5 kbp SalI-SacI fragment J cut down from plasmid pbet/chl. DNA Ligation Kit (Takara) using a ligase Use and it joins together by the system of reaction of all 50microl according to the manual of a kit. The strain into which plasmid pbet/chl/M2 (drawing 4) in which the betA structural gene part into which variation went by carrying out a transformation to Escherichia coli DH5alpha using this reaction mixture, and selecting by the chloramphenicol was inserted was introduced was obtained. This bacillus was cultivated and plasmid pbet/chl/M2 was refined.
[0050]
[Example 2] It is used for transformation vector pbet/chl/M2 to the manifestation (1) rice protoplast in the inside of the vegetation of a synthetic betA gene carrying out the transformation of the grass. That is, after suspending the protoplast of the grass origin to a liquid medium, impressing an electric pulse and introducing the vector concerned, it is the approach of cultivating by the culture medium containing a rice cultured cell, making a colony forming, and reproducing a plant body from the colony concerned (Shimamoto et al., Nature, 338:274-276, 1989).
[0051] The protoplast was prepared as follows. it produced from the full ripeness germ callus of a cultivation rice (form Japan fine) -- planting -- the suspension cell on three - the 5th after a patch -- 4% Cellulase RS and 1% MASEROZAIMU R-10 and 0.4M 30 degrees C was processed with the enzyme liquid (pH5.6) containing mannitol for 3 to 4 hours. After enzyme processing termination, it filtered, and the protoplasts which added the KMC liquid (0.118M potassium chloride, 0.0817M magnesium chloride, 0.085M calcium chloride, and pH6.0; refer to the above-mentioned reference) of an amount to the filtrate 4 times, carried out centrifugal separation except for the undigested object, and sedimented were collected, and KMC liquid washed twice further.
[0052] About the obtained protoplast, it is 70mM. Potassium chloride and 5mM A magnesium chloride and 0.4M It suspended so that it might become the buffer solution of pH5.8 containing mannitol and

0.1% MES in 8x106 pieces/[ml and].
[0053] In the plasmid vector 60microg/ml list containing the gene prepared as mentioned above to this suspension, as a promotor CaMV35S, The plasmid which has the terminator of a hygromycin phosphotransferase gene and NOS (nopaline synthase), or the CaMV origin as a foreign gene, For example, after adding pGL2 (276 338 : Nature, 274- 1989)60microg/ml and cooling for 5 minutes at 4 degrees C, it moved to the plastics cel which sterilized and the electric pulse of a direct current was impressed using the concurrency electrode. At that time, the initial voltage of 500 V/cm was applied using the 1000-micro F capacitor, and it was referred to as pulse width 30msec. After pulse impression and after cooling for 10 minutes at 4 degrees C, it mixed with the equivalent R2-/MS protoplast agarose culture medium (Mol.Gen.Genet., 206, 408, 1987), and was made to solidify so that it may become the thickness of 0.7mm. The cell density at this time was about 4x106 pieces/ml.
[0054] It put into the plate which is the diameter of 6cm into which the agarose containing the protoplast which carried out electric pulse processing was cut in adult magnitude 10mm, and R2 / 5ml of MS liquid protoplast culture media went, and the rice cultured cell of about 100mg (FW) was further put in as a nurse cell. Culture of a protoplast was cultivated under the dark condition, shaking slowly by rotation of 50 rpm for about ten days at about 29 degrees C.
[0055] This rice cultured cell was prepared as follows. The fine cell (diameter of 1mm) full of fission which exists the callus originating in the root of the rice of a seedling in 1-time ****** per week and the produced suspension culture cell in a liquid medium was used. The nurse cell was removed with KMC liquid after culture for ten days. Hygromycin B was added to the culture medium so that it might furthermore become in ml and 20-30microg /two - four days after culture, and it cultivated for two to three weeks.
[0056] Subsequently, this piece of agarose was placed and cultivated to R2 software agger culture medium (acetic acid (2, 4-D) 2 mg/l, 6% cane sugar, 0.25% agarose), the colony which became still larger was separately divided after two - four weeks, and it moved to R2 software agger culture medium. When this callus was moved to the R2-/MS playback culture medium (3% sorbitol, 2% cane sugar, 1% agarose, pH5.8) and it cultivated for three to ten weeks under 25 degrees C and the conditions of 2,000-4,000lux, the bud and the root appeared. It moved to the plastics box into which the R2-/MS playback culture medium was put, and was made to grow up to be a seedling in the place where the bud grew up into about 2cm. When it transplanted to the bar MYUKYU light pot and it was furthermore grown, the perfect transformation rice plant body which matured was obtained.
[0057] (2) DNA was extracted from the one section of the screening profit **** hygromycin tolerance callus of the transformed cell by the PCR method (Mol.Gen.Genet., 211, 27, 1988). Two calluses with a diameter of about 2-3mm are homogenized within a 1.5ml micro centrifugal tube with Resuspension buffer (20 mM Tris-HCl, 10mM EDTA) 250microl, and it is SDS 20% 20 mul In addition, 68 degrees C was warmed for 15 minutes. 7.5M Ammonium Acetate 150microl was added here, and it put on it for 30 minutes in Hikami. It is ethanol after 15,000 rpm., 4 degrees C, and a 15-minute alignment at long intervals and to supernatant liquid. In addition, centrifugal [of the 1ml] was again carried out on the same conditions, and DNA was settled. TE after washing obtained DNA by EtOH 70% and drying it It melted to 30micro (10 mM Tris-HCl (pH8.0), 1mM EDTA) of buffer solutions l.
[0058] This DNA was used for screening by the PCR method. The primer which has a base sequence equivalent to the base numbers 1098-1114 of the array number 1 was used for the primer which has a base sequence equivalent to the base numbers 9-26 of the array number 1 in a side primer, and the 5'5' side primer. The part of this primer is shown in drawing 5 . PCR Primer concentration every 1microM, 10 mM Tris-HCl (pH8.3), 1.5mM MgCl2 and 50mM KCl, 0.005% Tween 20 and 0.005% NP-40 and 0.001% Gelatin, dATP, dGTP, dCTP, and dTTP every 200microM, heat-resistant DNA polymerase (REPLITHERM Thermostable DNA Polymerase (EPISENTRE)) 5 units, Double DNA5microl prepared by the previous approach, and DNA thermal SAIKURA PJ1000 (PERKIN-ELMER CETUS) is used for the reaction mixture of all 50microl. The repeat reaction was carried out 30 to 35 times, and 94 degrees C, 1 minute ., 50 degree C, 2 minute ., 72 degree C, and the cycle that consists of 3 minutes were performed. When the PCR reaction product was analyzed by the agarose electrophoresis of a

conventional method, DNA amplified by the location of 1.1 kbp was looked at by the transformation callus in which plasmid pbet/chl/CM2 was included so that drawing 6 might see. As a result of screening screening many hygromycin tolerance calluses similarly, it was introduced into the callus at about 30% of effectiveness.

[0059] (3) All RNA was extracted from the transformant vegetation with which the gene of an overall length was introduced into the detection place of imprint-mRNA of the introduced gene by the Southern method (Analytical Biochem., 162, 156-159, 1987), and mRNA was extracted using Oligo-dT kit (TAKARA SHUZO). every -- mRNA 2microg -- NOZAN -- it analyzed by law (Thomas, P.et al., Proc.Natl.Acad.Sci., 77, 5201, 1980). The BamHI-NotI fragment of pbet/chl/M2 which includes as a probe the base sequence shown in the array number 1 was used. The location of this probe is shown in drawing 5 . Consequently, as shown in drawing 7 , with transformant vegetation, mRNA of 2.0 Kb expected from a betA gene was detected. In the transformant which introduced plasmid pbet/chl/M2 which added the alteration to the gene, although plasmid pbet/chl of a basis was introduced, a maximum of 11 times as many mRNA as this was detected.

[0060] (4) the betA protein in the sample which created the rabbit antibody to the detection Escherichia coli betA protein of the translation-betA protein of the introduced gene, and carried out the label by 35S by immunoprecipitation according to Blobel's and others approach -- condensing -- a law -- Western analysis was carried out according to the method, and the band of the betA protein origin of about 60 kDa(s) was detected (drawing 8).

[0061] (5) Detection transformant callus of the choline dehydrogenase activity of the translation product of the introduced gene A 46.5mM K-phosphate buffer solution (pH 7.4) and 10% It ground in the extract containing glycerol, 5mM dithiothreitol, 5 mM EDTA, 5 % polyvinyl pyrrolidone, 1microM PMSF (phenylmethane sulfonyl fluoride), and 1microM MIA (monoiodoacetic acid), centrifugal separation was carried out by 10,000rpm for 15 minutes, and supernatant liquid was obtained. What dialyzed this in the extract overnight and was obtained was made into the crude extract. After measuring the protein content of this crude extract and arranging the concentration between samples, As opposed to 400microl 91 mM K-phosphate buffer solution (pH 7.4) 300microl, 20mM KCN 50microl, 2mM DCPIP (2, 6-dichlorophenolindophenol sodium salt hydrate) 50microl, and 2.6mM PMS 50microl After mixing in addition and leaving it at 26 degrees C for 5 minutes, 0.73M The choline chloride was 50microl Added and reduction of absorption of 600 nm was measured for 2 minutes. When the activity value was calculated by setting to 1unit the amount of enzymes which returns DCPIP of 1microM in 1 minute, although plasmid pbet/chl of a basis was introduced, by the transformant which introduced plasmid pbet/chl/M2 which added the alteration to the gene, one a maximum of 10 times the choline dehydrogenase activity of this was detected (drawing 9).

[0062] (6) According to the approach (Plant Physiol.29 and 1315-1321 (1988)) of detection Arakawa and others of the betaine in transformant vegetation, a betaine is extracted from a rice transformation plant body, deposit this in the form of a periodate, dissolve in heavy water, and it is 500MHz. Are recording of a betaine was checked using 1 H-NMR. The quantum of the betaine content was carried out based on the amount of t-butanol added to coincidence as an internal standard. Although a betaine was not detected at all with the transformant vegetation which introduced plasmid pbet/chl of a basis, the betaine of 1-2micromoles/gFW (FW: fresh weight) was detected in the transformant which introduced plasmid pbet/chl/M2 which added the alteration to the gene (drawing 10). This is equivalent to 1/several [which accumulates a betaine / of a salt atmosphere strong barley] accumulated doses.

[0063]

[Effect of the Invention] The gene which carries out the code of the Escherichia coli betA protein of this invention carries out the code of the betA protein, except the poly(A) addition signal Mr. array and palindromic sequence which become the hindrance of the gene expression in the inside of a plant cell since, in a grass, it is high-discovered, the grass by which introduced this gene and the transformation was carried out produces and accumulates a betaine, and the usefulness as crops which have resistance in high salts stress or desiccation stress is expected.

[0064]

[Layout Table]
array number: -- die-length [of one array]: -- mold [of 1671 arrays]: -- number [of nucleic-acid chains]: -- double strand topology: -- a nucleic acid besides class: of a straight chain-like array .. 1..notation:CDS existence location:1668 description of expressing the description description of a thing array of having changed Genomic DNA partially The determined approach : E array ATG CAA TTC GAC TAC ATC ATC ATC GGT GCC GGG TCA GCG GGC AAC GTT 48 Met Gln Phe Asp Tyr Ile Ile Ile Gly Ala Gly Ser Ala Gly Asn Val1 5 10 15 CTC GCT ACC CGT CTG ACT GAA GAT CCG AAT ACC TCC GTG CTG CTG CTT 96 Leu Ala Thr Arg Leu Thr Glu Asp Pro Asn Thr Ser Val Leu Leu Leu 20 25 30 GAAGCG GGC GGC CCG GACTAT CGC TTT GAC TTC CGC ACC CAG ATG CCC 144 Glu Ala Gly Gly Pro Asp Tyr Arg Phe Asp PheArg ThrGln Met Pro 35 40 45 GCT GCC CTG GCATTC CCG CTA CAG GGT AAA CGC TAC AAC TGGGCC TAT 192 Ala Ala Leu Ala Phe Pro Leu Gln Gly Lys Arg Tyr Asn Trp Ala Tyr 50 55 60 GAA ACG GAA CCTGAA CCG TTT ATG AAC AAC CGC CGC ATG GAG TGC GGC 240 Glu Thr Glu Pro Glu Pro Phe Met Asn Asn Arg Arg Met Glu Cys Gly 65 70 75 80 CGC GGC AAG GGT CTG GGC GGC TCG TCG CTG ATC AAC GGC ATG TGC TAC 288 Arg Gly Lys Gly Leu Gly Gly Ser Ser Leu Ile Asn Gly Met Cys Tyr 85 90 95 ATC CGT GGC AAT GCG CTG GAT CTC GAT AAC TGG GCG CAA GAA CCC GGT 336 Ile Arg Gly Asn Ala Leu Asp Leu Asp AsnTrp Ala Gln Glu Pro Gly 100 105 110 CTG GAG AAC TGG AGC TAC CTC GAC TGC CTG CCC TACTAC CGC AAG GCC 384 Leu Glu Asn Trp Ser Tyr Leu Asp Cys Leu Pro Tyr TyrArg Lys Ala 115 120 125 GAG ACT CGC GAT ATG GGT GAA AAC GAC TAT CAC GGC GGT GAT GGC CCG 432 Glu Thr Arg Asp Met Gly Glu Asn Asp Tyr His Gly Gly Asp Gly Pro 130 135 140 GTG AGC GTC ACT ACC TCC AAA CCC GGC GTC AAT CCG CTG TTT GAA GCG 480 Val Ser Val Thr Thr Ser Lys Pro Gly Val Asn Pro LeuPhe Glu Ala 145 150 155 160 ATG ATT GAA GCG GGC GTG CAG GCG GGC TAC CCG CGC ACG GACGAT CT C 528Met Ile Glu Ala Gly-Val-Gln-Ala-Gly Tyr Pro Arg Thr Asp-Asp-Leu 165 170 175 AAC-GGT-TAT-CAG-CAG GAA GGT TTT GGT CCG-ATG-GAT-CGC-ACC-GTC-ACG 576Asn Gly Tyr Gln Gln Glu Gly Phe Gly Pro Met Asp Arg Thr Val Thr 180 185 190 CCG CAG GGC CGT CGC GCC AGC ACC GCG CGT GGC TAT CTC GAT CAG GCC 624 Pro Gln Gly Arg Arg Ala Ser Thr Ala Arg Gly Tyr LeuAsp Gln Ala 195 200 205 AAA TCG CGT CCT AAC CTG ACC ATT CGT ACT CAC GCTATGACC GAT CAC 672 Lys Ser Arg Pro Asn Leu Thr Ile Arg Thr His Ala Met Thr AspHis 210 215 220 ATC ATT TTT GAC GGC AAA CGC GCG GTG GGC GTC GAA TGG CTG GAA GGC 720 Ile Ile Phe Asp Gly Lys Arg Ala Val Gly Val Glu Trp Leu Glu Gly 225 230 235 240 GAC AGC ACC ATC CCA ACC CGC GCA ACG GCC AAC AAA GAA GTG CTG TTA 768 Asp Ser Thr Ile Pro Thr Arg Ala Thr Ala Asn Lys Glu Val Leu Leu 245 250 255 TGT GCA GGC GCG ATT GCC TCA CCG CAG ATC CTG CAACGC TCC GGC GTC 816 Cys Ala Gly Ala Ile Ala Ser Pro Gln Ile Leu Gln Arg Ser Gly Val 260 265 270 GGC AAC GCT GAA CTG CTG GCG GAG TTT GAT ATT CCG CTG GTG CAT GAA 864 Gly Asn Ala Glu Leu Leu Ala Glu Phe AspIle Pro Leu Val His Glu 275 280 285 TTACCC GGC GTC GGC GAA AAT CTT CAG GAT CATCTG GAG ATG TAT CTG 912 Leu Pro Gly Val Gly Glu Asn Leu Gln Asp His Leu GluMet Tyr Leu 290 295 300 CAA TAT GAG TGC AAA GAA CCG GTT TCC CTC TAC CCT GCC CTG CAG TGG960 Gln Tyr Glu Cys Lys Glu Pro Val Ser Leu Tyr Pro Ala Leu Gln Trp 305 310 315 320 TGG AAC CAG CCG AAG ATC GGC GCG GAG TGG CTG TTC GGC GGC ACC GGC 1008 Trp Asn Gln Pro Lys Ile Gly Ala Glu Trp Leu Phe Gly Gly Thr Gly 325 330 335 GTC GGC GCCAGC AAC CAC TTC GAG GCG GGC GGC TTC ATC CGC AGC CGC 1056 Val Gly Ala Ser Asn His Phe Glu Ala Gly Gly Phe Ile Arg Ser Arg 340 345 350 GAG-GAG-TTC-GCG-TGG CCG AAC ATC CAG TAC-CAC-TTC-CTG-CCG-GTC-GCG 1104Glu Glu Phe Ala Trp Pro Asn Ile Gln-Tyr-His-Phe-Leu-Pro-Val-Ala 355 360 365 ATC AAC TAC AAC GGC TCG AAC GCC GTG AAG-GAG-CACGGC TTC CAG TGC 1152 Ile Asn Tyr Asn Gly Ser Asn Ala Val LysGlu His Gly Phe Gln Cys 370 375 380CAC GTC GGC TCA ATG CGC TCG CCA AGC CGT GGG CAT GTG CGG ATT AAA 1200 His Val Gly Ser Met Arg Ser Pro Ser Arg Gly His Val Arg Ile Lys385 390 395 400 TCC CGCGACCCG CAC CAG CAT CCG GCG ATT CTG TTT AAC TAC ATG TCG 1248 Ser Arg Asp Pro His Gln His Pro Ala Ile Leu Phe Asn Tyr Met Ser 405 410 415 CAC GAG CAGGAC TGG CAG GAG TTC CGC GAC GCA ATT CGC ATC ACC CGC 1296 His Glu Gln Asp Trp Gln Glu Phe

Arg Asp AlaIle Arg Ile Thr Arg 420 425 430 GAG ATC ATG CAT CAA CCC GCG CTG GAT CAG TAT CGT GGC CGC GAA ATC 1344 Glu Ile Met His Gln Pro Ala Leu Asp Gln Tyr Arg Gly Arg Glu Ile 435 440 445 AGCCCC GGT GTC GAA TGC CAG ACG GAT GAA CAGCTC GAT GAG TTC GTG 1392 Ser Pro Gly Val Glu Cys Gln Thr Asp Glu Gln Leu Asp Glu Phe Val 450 455 460 CGT AAC CAC GCC GAA ACC GCC TTC CAT CCG TGC GGT ACCTGC AAA ATG1440 Arg Asn His Ala Glu Thr Ala Phe His Pro Cys Gly Thr Cys Lys Met 465 470 475 480 GGT TAC GAC GAG ATG TCC GTG GTT GAC GGC GAA GGC CGC GTA CAC GGG 1488 Gly Tyr Asp Glu Met Ser Val Val Asp Gly Glu Gly Arg Val His Gly 485 490 495 TTA GAA GGCCTG CGT GTG GTG GAT GCG TCG ATT ATG CCG CAG ATT ATC 1536 Leu Glu Gly Leu Arg Val Val Asp Ala Ser Ile Met Pro Gln Ile Ile 500 505 510 ACC GGG AAT TTG AAC GCC ACG ACA ATT ATG ATTGGC GAG AAA ATA GCG 1584 Thr Gly Asn Leu Asn Ala Thr Thr Ile Met Ile Gly Glu Lys Ile Ala 515 520 525 GATATG ATT CGT GGA CAG GAA GCG CTG CCG AGGAGC ACG GCG GGA TAT 1632 Asp Met Ile Arg Gly Gln Glu Ala Leu Pro Arg Ser Thr Ala Gly Tyr 530 535 540 TTT GTG GCA AAT GGG ATG CCG GTG AGA GCG AAA AAA TGA 1671 Phe Val Ala Asn Gly Met Pro Val Arg Ala Lys Lys 545 550 555 [0065] array number: -- die-length [of two arrays]: -- mold [of 1710 arrays]: -- number [of nucleic-acid chains]: -- double strand topology: -- class [of straight chain-like array]: -- Genomic DNA origin living thing name: -- Escherichia coli (Escherichia coli)
stock name: -- notation:CDS existence location: showing the description description of K-10 array -- approach: which determined 7..1674 description -- E array GGATCC ATG CAA TTT GAC TAC ATC ATT ATT GGT GCC GGC TCA GCC GGC 48 Met Gln Phe Asp Tyr Ile Ile Ile Gly Ala Gly Ser Ala Gly 1 5 10 AAC GTT CTC GCT ACC CGT CTG ACT GAA GAT CCG AAT ACC TCC GTG CTG 96 Asn Val Leu Ala Thr Arg Leu Thr Glu AspPro Asn Thr Ser Val Leu 15 20 25 30 CTG CTTGAA GCG GGC GGC CCG GAC TAT CGC TTTGAC TTC CGC ACC CAG 144 Leu Leu Glu Ala Gly Gly Pro Asp Tyr Arg Phe Asp Phe Arg Thr Gln 35 40 45 ATG CCC GCT GCC CTG GCA TTC CCG CTA CAGGGT AAA CGC TAC AAC TGG 192 Met Pro Ala Ala Leu Ala Phe Pro Leu Gln Gly Lys Arg Tyr Asn Trp 50 55 60 GCC TAT GAA ACG GAA CCT GAA CCG TTT ATG AAT AAC CGC CGC ATG GAG 240 Ala Tyr Glu Thr Glu Pro Glu Pro Phe Met Asn Asn Arg Arg Met Glu 65 70 75 TGC GGA CGC GGT AAA GGT CTG GGT GGA TCG TCG CTG ATC AAC GGC ATG 288 Cys Gly Arg Gly Lys Gly Leu Gly Gly Ser SerLeu Ile Asn Gly Met 80 85 90 TGC TAC ATCCGT GGC AAT GCG CTG GAT CTC GAT AAC TGG GCG CAA GAA336 Cys Tyr Ile Arg Gly Asn Ala Leu Asp Leu Asp Asn Trp Ala Gln Glu 95 100 105 110 CCC GGT CTG GAG AAC TGG AGC TAC CTC GAC TGC CTG CCC TAC TAC CGC 384 Pro Gly Leu Glu Asn Trp Ser Tyr Leu Asp Cys Leu Pro Tyr Tyr Arg 115 120 125 AAG GCC GAGACT CGC GAT ATG GGT GAA AAC GAC TAT CAC GGC GGT GAT 432 Lys Ala Glu Thr Arg Asp Met Gly Glu Asn Asp Tyr HisGly Gly Asp 130 135 140 GGC CCG GTG AGC GTC ACT ACC TCC AAA CCC GGCGTC AAT CCG CTG TTT 480 Gly Pro Val Ser Val Thr Thr Ser Lys Pro Gly Val Asn Pro Leu Phe 145 150 155 GAAGCG ATG ATT GAA GCG GGC GTG CAG GCG GGC TAC CCG CGC ACG GAC 528 Glu Ala Met Ile Glu Ala Gly Val Gln Ala Gly Tyr Pro Arg Thr Asp 160 165 170 GAT CTC AAC GGT TAT CAG CAG GAA GGT TTT GGT CCG ATGGAT CGC ACC576 Asp Leu Asn Gly Tyr Gln Gln Glu Gly Phe Gly Pro Met Asp ArgThr 175 180 185 190 GTC ACG CCGCAG GGC CGT CGC GCC AGC ACC GCG CGT GGC-TAT-CTC-GAT 624Val Thr Pro Gln Gly-Arg-Arg-Ala-Ser Thr Ala Arg Gly Tyr-Leu-Asp 195 200 205 CAG-GCC-AAA-TCG-CGT CCT AAC CTG ACC ATT-CGT-ACT-CAC-GCT-ATG-ACC 672Gln Ala Lys Ser Arg-ProAsn Leu Thr IleArg Thr His Ala Met Thr 210 215 220 GAT CAC ATC ATT TTT GAC GGC AAA CGC GCG GTG GGC GTC GAA TGG CTG 720 Asp His Ile Ile Phe Asp Gly Lys Arg Ala Val Gly ValGlu Trp Leu 225 230 235 GAA GGC GAC AGC ACC ATC CCA ACC CGC GCA ACG GCC AAC AAA GAA GTG 768 Glu Gly Asp Ser Thr Ile Pro Thr Arg Ala Thr Ala Asn Lys GluVal 240 245 250 CTG TTA TGT GCA GGC GCG ATT GCC TCA CCG CAG ATC CTG CAA CGC TCC 816 Leu Leu Cys Ala Gly Ala Ile Ala Ser Pro Gln Ile Leu Gln Arg Ser 255 260 265 270 GGC GTC GGC AAC GCT GAA CTG CTG GCG GAG TTT GAT ATT CCG CTG GTG 864 Gly Val Gly Asn Ala Glu Leu Leu Ala Glu Phe Asp Ile Pro Leu Val 275 280 285 CAT GAA TTA CCC GGC GTC GGC GAA AAT CTT CAG GATCAT CTG GAG ATG 912 His Glu Leu Pro Gly Val Gly Glu Asn Leu Gln Asp His Leu Glu

Met 290 295 300 TAT CTG CAA TAT GAG TGC AAA GAA CCG GTT TCC CTC TAC CCT GCC CTG 960 Tyr Leu Gln Tyr Glu Cys Lys Glu Pro ValSer Leu Tyr Pro Ala Leu 305 310 315 CAGTGG TGG AAC CAG CCG AAA ATC GGT GCG GAGTGG CTG TTT GGC GGC 1008 Gln Trp Trp Asn Gln Pro Lys Ile Gly Ala Glu Trp LeuPhe Gly Gly 320 325 330 ACT GGC GTT GGT GCC AGC AAC CAC TTT GAA GCA GGT GGA TTT ATT CGC1056 Thr Gly Val Gly Ala Ser Asn His Phe Glu Ala Gly Gly Phe Ile Arg 335 340 345 350 AGC CGT GAG GAA TTT GCG TGG CCG AAT ATT CAG TAC CAT TTC CTG CCA 1104 Ser Arg Glu Glu Phe Ala Trp Pro Asn Ile Gln Tyr His Phe Leu-Pro 355 360 365 GTA-GCG-ATT-AAC-TAT AAC GGC TCG AAT GCA-GTG-AAA-GAG-CAC-GGT-TTC 1152Val Ala Ile Asn Tyr Asn Gly Ser Asn-Ala-Val-Lys-Glu-His-Gly-Phe 370 375 [380] CAG TGC CAC GTC GGC TCA ATG CGC TCG CCA AGC CGT GGG CAT GTG CGG 1200 Gln Cys His Val Gly Ser Met Arg Ser Pro Ser Arg Gly His ValArg 385 390 395 ATT AAA TCC CGC GAC CCG CACCAG CAT CCG GCG ATT CTG TTT AAC TAC 1248 Ile Lys Ser Arg Asp Pro His Gln His Pro Ala Ile Leu Phe Asn Tyr 400 405 410 ATG TCG CAC GAG CAG GAC TGG CAG GAG TTC CGC GAC GCA ATT CGC ATC 1296 Met Ser His Glu Gln Asp Trp Gln Glu Phe Arg Asp Ala Ile Arg Ile 415 420 425 430 ACC CGC GAG ATC ATG CAT CAA CCC GCG CTG GAT CAG TAT CGT GGC CGC 1344 Thr Arg Glu Ile Met His Gln Pro Ala Leu Asp Gln Tyr Arg Gly Arg 435 440 445 GAA ATC AGC CCC GGT GTC GAA TGC CAG ACG GAT GAA CAG CTC GAT GAG 1392 Glu Ile Ser Pro Gly Val Glu Cys Gln Thr Asp GluGln Leu Asp Glu 450 455 460 TTC GTG CGT AAC CAC GCC GAA ACC GCC TTC CAT CCG TGC GGT ACC TGC 1440 Phe Val Arg Asn His Ala Glu Thr Ala Phe His Pro Cys Gly Thr Cys 465 470 475 AAAATG GGT TAC GAC GAG ATG TCC GTG GTTGACGGC GAA GGC CGC GTA 1488 Lys Met Gly Tyr Asp Glu Met Ser Val Val Asp Gly Glu Gly Arg Val 480 485 490 CAC GGG TTA GAA GGC CTG CGT GTG GTG GAT GCG TCG ATT ATG CCG CAG1536 His Gly Leu Glu Gly Leu Arg Val Val Asp Ala Ser Ile Met Pro Gln 495 500 505 510 ATT ATC ACC GGG AAT TTG AAC GCC ACG ACA ATT ATG ATT GGC GAG AAA 1584 Ile Ile Ile Thr Gly Asn Leu Asn Ala Thr Thr Ile Met Ile Gly Glu Lys 515 520 525 ATA GCG GATATG ATT CGT GGA CAG GAA GCG CTG CCG AGG AGC ACG GCG 1632 Ile Ala Asp Met Ile Arg Gly Gln Glu Ala LeuPro Arg Ser Thr Ala 530 535 540 GGA TAT TTT GTG GCA AAT GGG ATG CCG GTG AGA GCG AAA AAA 1674 Gly Tyr Phe Val Ala Asn Gly Met Pro Val Arg Ala Lys Lys 545 550 555 TGAGTCGTGA TGTGAACTAA CGCAGGAACC GAGCTC 1710 [0066] array number: -- die-length [of three arrays]: -- mold [of 556 arrays]: -- amino acid topology: -- class [of straight chain-like array]: -- protein array Met GlnPhe Asp Tyr Ile Ile Ile Gly Ala Gly Ser Ala Gly Asn Val One 5 10 15 Leu Ala Thr Arg Leu Thr Glu Asp Pro Asn Thr Ser Val Leu Leu Leu 20 25 30 Glu Ala Gly Gly Pro Asp Tyr Arg Phe Asp Phe Arg Thr Gln Met Pro 35 40 45 Ala Ala Leu Ala Phe Pro Leu Gln Gly Lys Arg Tyr Asn Trp Ala Tyr 50 55 60 Glu Thr Glu Pro Glu Pro Phe Met Asn Asn Arg Arg Met Glu Cys Gly 65 70 75 80 Arg GlyLys Gly Leu Gly Gly Ser Ser Leu Ile Asn Gly Met Cys Tyr 85 90 95 Ile Arg Gly Asn AlaLeu Asp LeuAsp Asn Trp Ala Gln Glu Pro Gly 100 105 110 Leu Glu Asn Trp Ser Tyr Leu Asp Cys Leu Pro Tyr Tyr Arg Lys Ala 115 120 125 Glu Thr Arg Asp Met Gly Glu Asn Asp Tyr His Gly Gly Asp Gly Pro 130 135 140 Val Ser Val Thr Thr Ser Lys Pro Gly Val Asn Pro Leu Phe Glu Ala 145 150 155 160 Met Ile Glu Ala Gly Val Gln Ala Gly TyrPro Arg Thr Asp Asp Leu 165 170175 AsnGly Tyr Gln Gln Glu Gly PheGly Pro Met Asp Arg Thr Val Thr 180 185 190 Pro Gln Gly Arg Arg Ala SerThr Ala Arg Gly Tyr Leu Asp Gln Ala 195 200 205 Lys Ser Arg Pro Asn Leu Thr Ile Arg Thr His Ala Met Thr Asp His 210 215 220 Ile Ile Phe Asp Gly Lys Arg Ala ValGly Val Glu Trp Leu Glu Gly 225 230 235 240 Asp Ser Thr Ile Pro Thr Arg Ala ThrAla Asn Lys Glu Val Leu Leu 245 250 255 Cys Ala Gly AlaIleAla Ser Pro Gln Ile Leu Gln Arg Ser Gly Val 260 265270 Gly Asn Ala Glu LeuLeuAla Glu Phe Asp Ile Pro Leu Val His Glu 275 280285 Leu Pro Gly Val Gly Glu Asn Leu Gln Asp His Leu Glu Met Tyr Leu 290 295 300 GlnTyr Glu Cys Lys Glu ProVal Ser Leu Tyr Pro Ala Leu Gln Trp 305 310 315 320 Trp Asn Gln Pro Lys Ile Gly Ala Glu-Trp-Leu-Phe-Gly-Gly-Thr-Gly 325 330 335 Val-Gly-Ala-Ser-Asn His Phe Glu Ala Gly-Gly-Phe-Ile-Arg-Ser-Arg 340 345 350 Glu Glu Phe Ala Trp Pro Asn Ile Gln Tyr His Phe Leu Pro Val Ala 355 360 365 Ile Asn Tyr Asn Gly Ser Asn Ala Val Lys Glu His Gly Phe Gln Cys 370 375 380 His ValGly Ser Met Arg Ser Pro Ser Arg Gly His Val Arg Ile Lys 385 390 395 400 Ser Arg Asp Pro His Gln His Pro Ala Ile Leu Phe Asn Tyr Met Ser 405 410

415 His Glu Gln Asp Trp Gln Glu Phe Arg Asp Ala Ile Arg Ile Thr Arg 420 425 430 Glu Ile Met His Gln Pro Ala Leu Asp Gln Tyr Arg Gly Arg Glu Ile 435 440 445 Ser Pro Gly Val Glu Cys Gln Thr Asp Glu Gln Leu Asp Glu Phe Val 450 455 460 Arg Asn His Ala Glu Thr Ala Phe His Pro Cys Gly Thr Cys Lys Met 465 470 475 480 Gly Tyr Asp Glu Met Ser Val Val Asp Gly Glu Gly Arg Val His Gly 485 490 495 Leu Glu Gly Leu Arg Val Val Asp Ala Ser Ile Met Pro Gln Ile Ile 500 505 510 Thr Gly Asn Leu Asn Ala Thr Thr Ile Met Ile Gly Glu Lys Ile Ala 515 520 525 Asp Met Ile Arg Gly Gln Glu Ala Leu Pro Arg Ser Thr Ala Gly Tyr 530 535 540 Phe Val Ala Asn Gly Met Pro Val Arg Ala Lys Lys 545 550 555 [0067] array number: -- die-length [of four arrays]: -- mold [of 306 arrays]: -- number [of nucleic-acid chains]: -- double strand topology: -- class [of straight chain-like array]: -- notation:CDS existence location: showing the description description of a synthetic DNA array -- approach: which determined 1..306 description -- E array AGGGTGATGG GATCCATGCA ATTCGACTAC ATCATCATCG GTGCCGGGTC AGCGGGCAAC 60 GTTCTCGCTA CCCGTCTGAC TGAAGATCCG AATACCTCCG TGCTGCTGCT TGAAGCGGGC 120 GGCCCGGACT ATCGCTTTGA CTTCCGCACC CAGATGCCCG CTGCCCTGGC ATTCCCGCTA 180 CAGGGTAAAC GCTACAACTG GGCCTATGAA ACGGAACCTG AACCGTTTAT GAACAACCGC 240 CGCATGGAGT GCGGCCGCGG CAAGGGTCTG GGCGGCTCGT CGCTGATCAA CGGCATGTGC 300TACATC 306 [0068] array number: -- die-length [of five arrays]: -- mold [of 238 arrays]: -- number [of nucleic-acid chains]: -- double strand topology: -- the shape of a straight chain class [of array]: -- notation:CDS existence location: showing the description description of a synthetic DNA array -- approach: which determined 1..238 description -- E array GGTTTCCCTC TACCCTGCCC TGCAGTGGTG GAACCAGCCG AAGATCGGCG CGGAGTGGCT 60 GTTCGGCGGC ACCGGCGTCG GCGCCAGCAA CCACTTCGAG GCGGGCGGCT TCATCCGCAG 120 CCGCGAGGAG TTCGCGTGGC CGAACATCCA GTACCACTTC CTGCCGGTCGCGATCAACTA 180 CAACGGCTCG AACGCCGTGA AGGAGCACGG CTTCCAGTGC CACGTCGGCT CAATGCGC 238[0069] array number: -- die-length [of six arrays]: -- mold [of 63 arrays]: -- number [of nucleic-acid chains]: -- double strand topology: -- class [of straight chain-like array]: -- notation:CDS existence location: showing the description description of a synthetic DNA array -- approach:E array ATGGGATCCA TGCAATTCGA CTACATCATC ATCGGTGCCG GGTCAGCGGG CAACGTTCTC which determined 1..63 description 60GCT 63 [0070] array number: -- die-length [of seven arrays]: -- mold [of 63 arrays]: -- number [of nucleic-acid chains]: -- double strand topology: -- class [of straight chain-like array]: -- notation:CDS existence location: showing the description description of a synthetic DNA array -- approach:E array TTGATCAGCG ACGAGCCGCC CAGACCCTTG CCGCGGCCGC ACTCCATGCG GCGGTTGTTC which determined 1..63 description 60ATA 63 [0071] array number: -- die-length [of eight arrays]: -- mold [of 85 arrays]: -- number [of nucleic-acid chains]: -- double strand topology: -- class [of straight chain-like array]: -- notation:CDS existence location: showing the description description of a synthetic DNA array -- approach:E array CTCTACCCTG CCCTGCAGTG GTGGAACCAG CCGAAGATCG GCGCGGAGTG GCTGTTCGGC which determined 1..85 description 60 GGCACCGGCG TCGGCGCCAG CAACC 85 [0072] array number: -- die-length [of nine arrays]: -- mold [of 85 arrays]: -- number [of nucleic-acid chains]: -- double strand topology: -- class [of straight chain-like array]: -- notation:CDS existence location: showing the description description of a synthetic DNA array -- approach:E array TCGGCGCCAG CAACCACTTC GAGGCGGGCG GCTTCATCCG CAGCCGCGAG GAGTTCGCGT which determined 1..85 description 60 GGCCGAACAT CCAGTACCAC TTCCT 85 [0073] array number: -- die-length [of ten arrays]: -- mold [of 85 arrays]: -- number [of nucleic-acid chains]: -- double strand topology: -- class [of straight chain-like array]: -- notation:CDS existence location: showing the description description of a synthetic DNA array -- approach:E array TGGAGCCGAC GTGGCACTGG AAGCCGTGCT CCTTCACGGC GTTCGAGCCG TTGTAGTTGA which determined 1..85 description 60 TCGCGACCGG CAGGAAGTGG TACTG 85

[Translation done.]

* NOTICES *

1.This document has been translated by computer. So the translation may not reflect the original precisely.
2.**** shows the word which can not be translated.
3.In the drawings, any words are not translated.

CLAIMS

[Claim(s)]
[Claim 1] The gene which has the base sequence by which the poly(A) addition signal Mr. array and the palindromic sequence were removed from the base sequence of the gene which carries out the code of the natural mold Escherichia coli betA protein, and carries out the code of the Escherichia coli betA protein.
[Claim 2] The gene according to claim 1 as which said poly(A) addition signal Mr. array is chosen from ATTATT, AATAAC, TTTATT, AATATT, ATTAAC, and TATAAC.
[Claim 3] The gene according to claim 1 said whose palindromic sequence is TGCCGGCTCAGCCGGCA.
[Claim 4] ATTATT which is a poly(A) addition signal Mr. array in the base sequence of the gene which carries out the code of the natural mold betA protein is a gene according to claim 1 to which TTTATT is characterized by permuting ATTAAC by ATCAAC, and for TATAAC being permuted for AATATT by TACAAC by AACATC, and permuting palindromic sequence TGCCGGCTCAGCCGGCA by TGCCGGGTCAGCGGGCA by AATAAC at TTCATC at AACAAC to ATCATC.
[Claim 5] The gene according to claim 4 characterized by what is expressed with the base sequence of a publication to the array number 1 of an array table.
[Claim 6] The vector characterized by introducing the gene of a publication into any 1 term of claims 1-5.
[Claim 7] The grass which was made to reproduce a plant body and was obtained from this colony after introducing the vector according to claim 6 into the protoplast of the grass origin and making a colony form from this protoplast.
[Claim 8] The manufacture approach of the grass which produces the Escherichia coli betA protein which cultivates by the culture medium containing a rice cultured cell, is made to form a colony, and is characterized by reproducing ******** from this colony after suspending the protoplast of a vector according to claim 6 and the grass origin in a liquid medium, impressing an electric pulse and introducing this vector.

[Translation done.]

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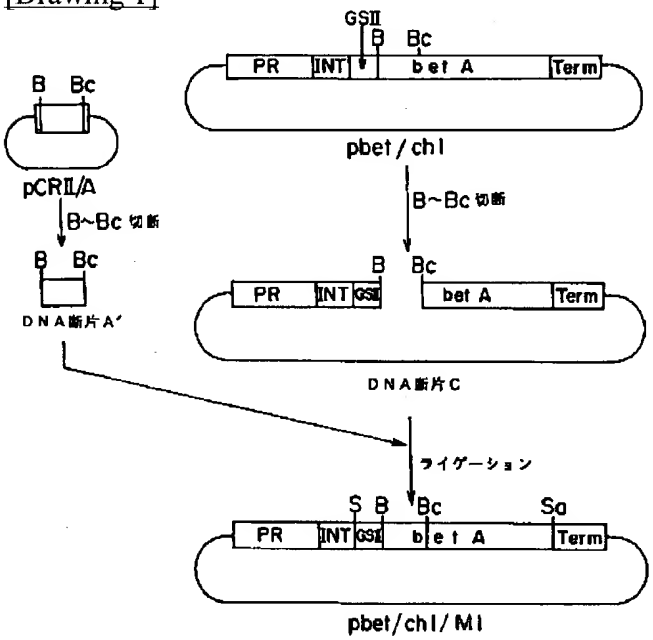
DRAWINGS

[Drawing 1]



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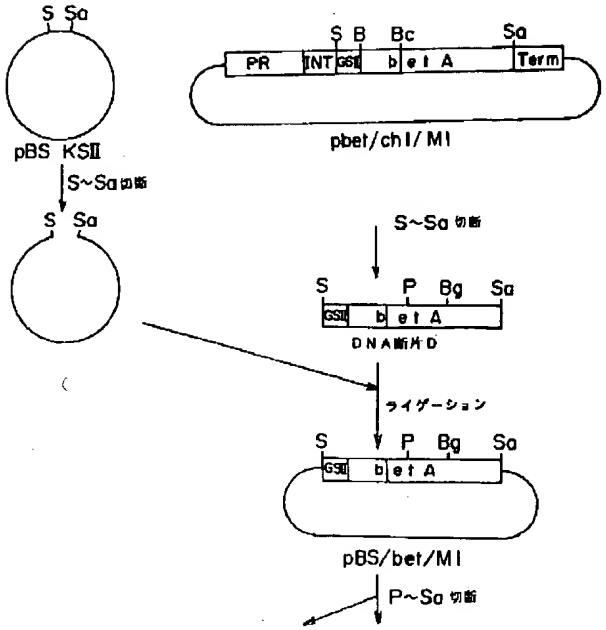

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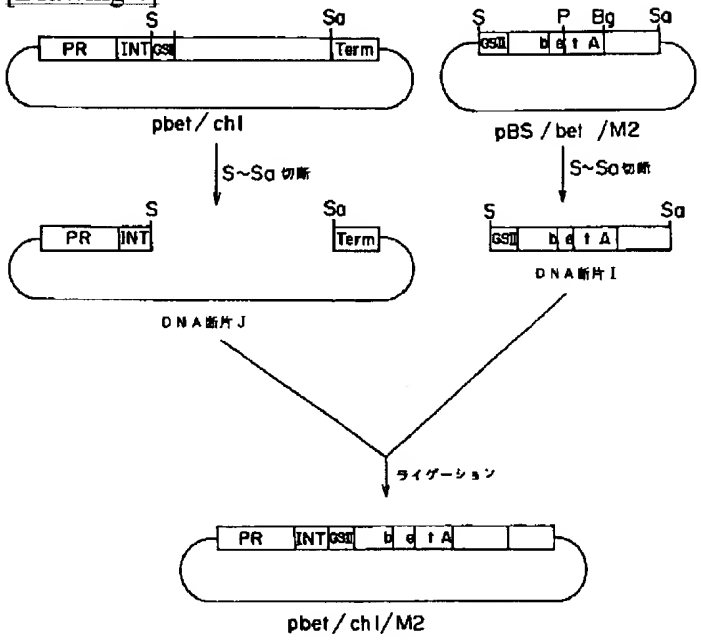

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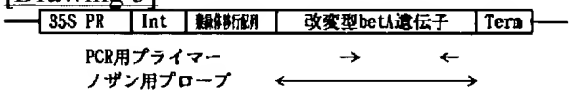
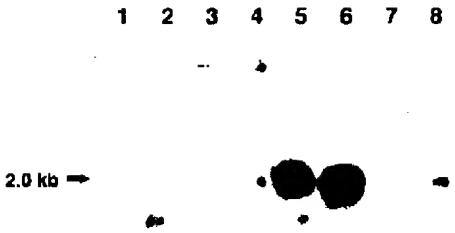


35S PR ：カリフラワーモザイクウイルス35Sプロモーター
Int ：ヒマカタラーゼ遺伝子第1イントロン
Term ：ノパリンシンターゼ遺伝子ターミネーター

[Drawing 7]



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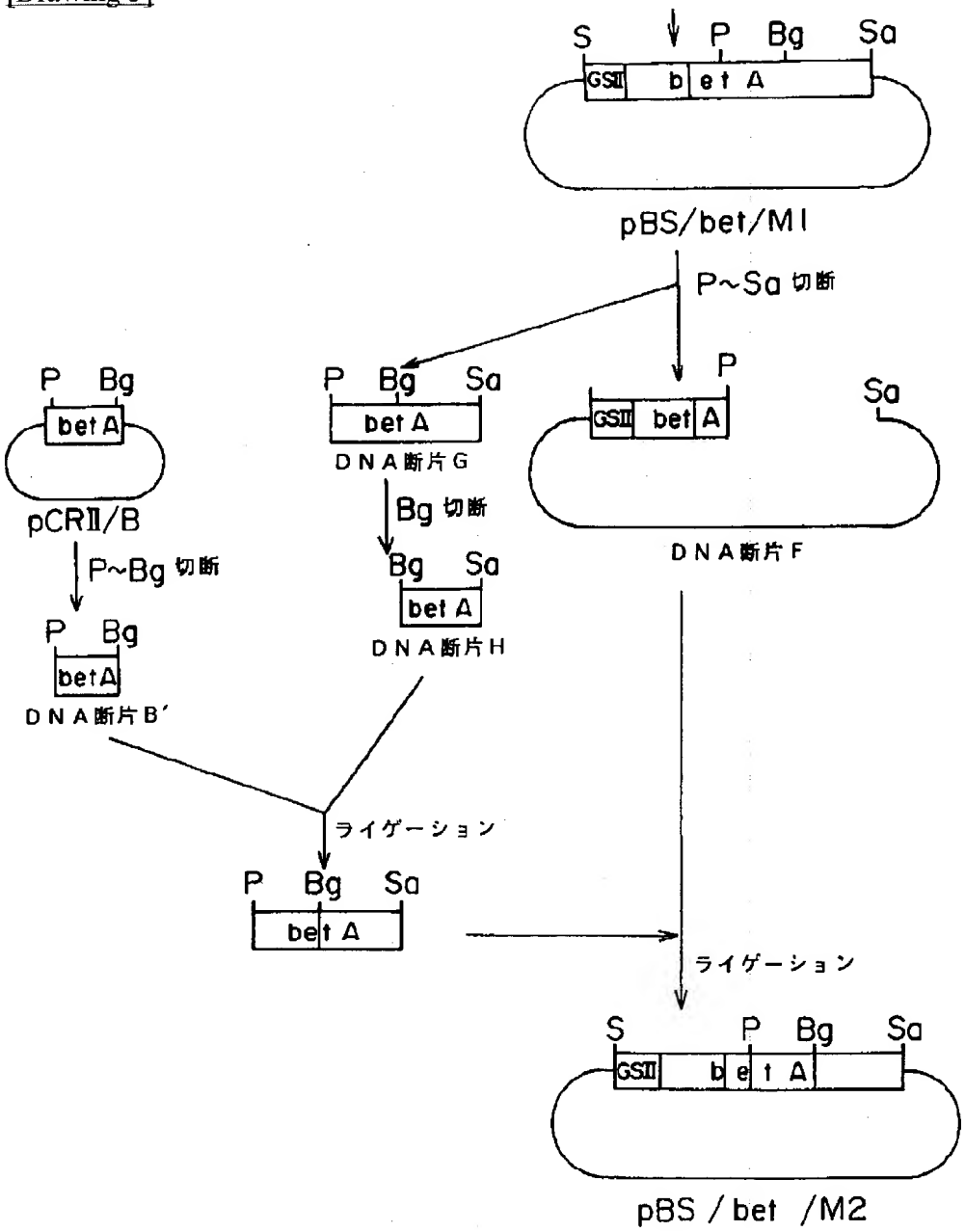
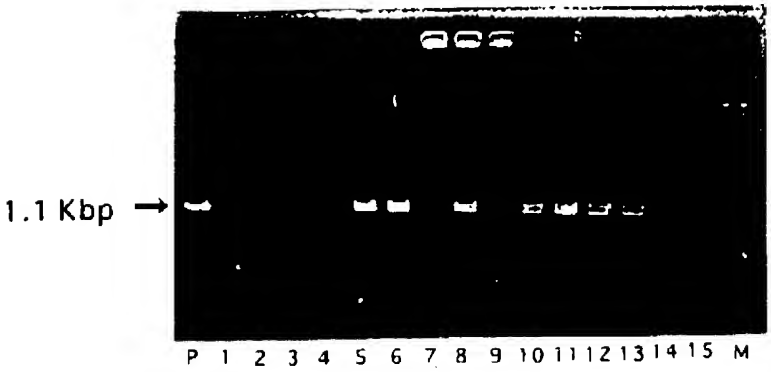


[Drawing 6]



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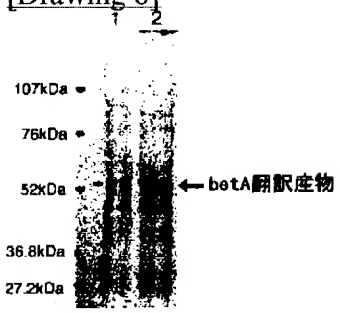
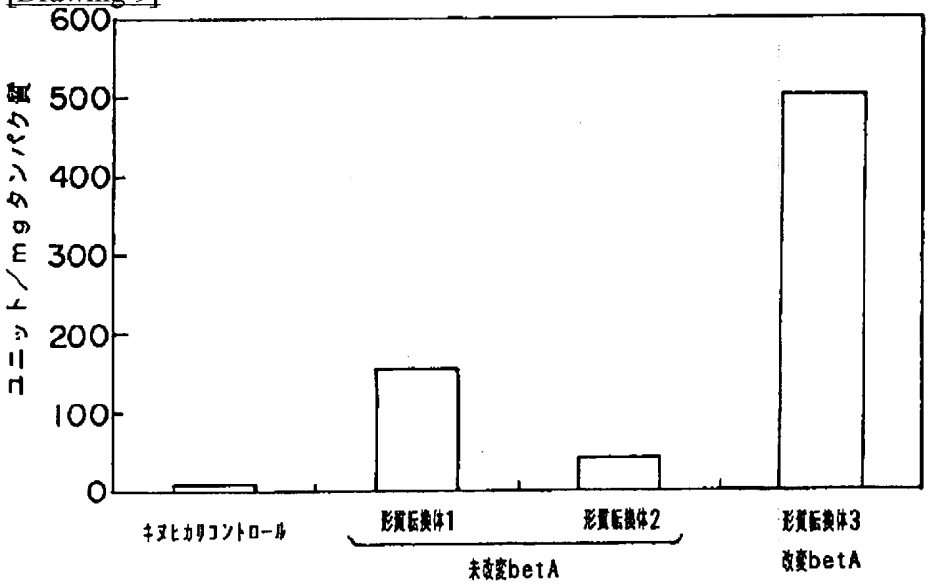
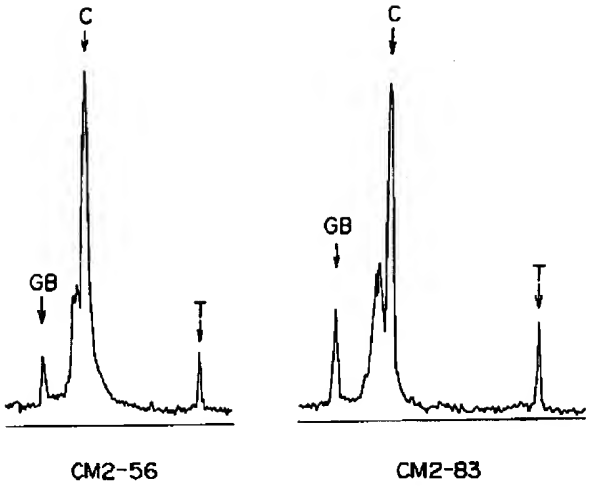


[Drawing 9]



[Drawing 10]



[Translation done.]